



# Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.)

Y. Fracheboud<sup>1</sup>, P. Haldimann<sup>2</sup>, J. Leipner and P. Stamp

Institute of Plant Sciences, Swiss Federal Institute of Technology of Zurich, Universitätstrasse 2, CH-8092 Zurich, Switzerland

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## Abstract

The possibility of using quenching analysis of chlorophyll *a* fluorescence as a selection tool for improving the cold tolerance of maize was investigated in six genotypes differing greatly in the ability to develop a competent photosynthetic apparatus at low temperature. Upon gradual cooling, measurements of the quantum yield of electron transport ( $\Phi_{PSII}$ ) indicated that leaves of tolerant genotypes, that developed at suboptimal temperature (15 °C), maintained higher rates of electron transport than leaves of sensitive genotypes. This difference was largely due to the ability of the tolerant plants to keep higher efficiency of excitation energy capture by open photosystem II reaction centres ( $F_v/F_m$ ). The absence of genotypic differences in leaves that developed at optimal temperature indicates that the trait is not expressed constitutively, but relies on adaptation mechanisms. Furthermore, the genotypic difference was not expressed under increasing illumination at 15 °C and 25 °C suggesting that the trait is also low-temperature-specific and is not expressed solely in response to increasing excess light energy. Applying the method to flint and dent breeding population led to a substantial increase (up to 31%) in the photosynthetic capacity of hybrids between selected  $F_3$  inbreeding families grown at suboptimal temperature, demonstrating that the method is an efficient selection tool for improving the cold tolerance of maize through breeding.

Key words: Chlorophyll fluorescence, cold tolerance, photosynthesis, breeding, maize.

## Introduction

It is necessary to improve the adaptation of maize to low temperature, because over the past 50 years, the cultiva-

tion of maize has been extended to areas in cooler regions. It has become a major crop in northern regions where its high temperature requirement is not always fulfilled. Suboptimal temperature during spring results in decreased productivity (Carr and Hough, 1978) and poor yield stability (Stamp, 1986). Amongst the various effects of low temperature on the physiology of maize (Stamp, 1984), the high susceptibility of the photosynthetic apparatus to low temperature is considered to be of particular importance (Hayden and Baker, 1990; Baker, 1994). The photosynthetic apparatus of maize is known to be highly sensitive to low temperature-induced photoinhibition (Long *et al.*, 1983; Nie *et al.*, 1992). Leaves that develop at a temperature of 15 °C or below are also characterized by a very low photosynthetic capacity (Nie *et al.*, 1992; Haldimann *et al.*, 1996), altered leaf pigment composition (Haldimann *et al.*, 1995; Haldimann, 1998) and impaired chloroplast development (Robertson *et al.*, 1993). Improved cold tolerance of the photosynthetic apparatus of maize may, therefore, contribute substantially to improving the performance of the crop in temperate regions by increasing early vigour and extending the culture period.

In the past 15 years *in vivo* chlorophyll fluorescence measurements are commonly used to study the functioning of the photosynthetic apparatus. In particular, quenching analysis using the saturation pulse technique (Schreiber *et al.*, 1986) provides semi-quantitative information about photochemistry in intact leaves. This method has been used extensively to investigate the response of plants to environmental stress, including the effects of low temperature on the photosynthetic apparatus of maize both in a controlled environment (Havaux, 1987; Haldimann *et al.*, 1996) and in the field (Andrews *et al.*, 1995). The use of chlorophyll fluorescence measurements as a screening method for cold tolerance has

<sup>1</sup> To whom correspondence should be addressed. Fax: +41 1 632 11 43. E-mail: yvan.fracheboud@ipw.agrl.ethz.ch

<sup>2</sup> Present address: Laboratoire de bioénergétique, Université de Genève, Ch. des Embouches 10, 1254 Jussy-Lullier, Switzerland.

already been investigated in experiments in which genotypes were compared (Hetherington *et al.*, 1983; Havaux, 1987; Schapendonk *et al.*, 1989; Dolstra *et al.*, 1994). The genotypic variability of plants developed at suboptimal temperature has, however, not been investigated. Results of several studies indicate that the photosynthetic apparatus of maize can adapt to suboptimal growth temperature and become more tolerant to severe chilling stress. It has been shown, for example, that such leaves recover faster from photoinhibition (Haldemann *et al.*, 1996), are more tolerant to chill-induced photo-oxidation and contain greater amounts of certain leaf antioxidants (Leipner *et al.*, 1997). Thus, it is likely that some of the genotypic differences related to low temperature tolerance may be detectable only in leaves that develop at low temperature.

The present study analyses the temperature response of chlorophyll fluorescence quenching in maize leaves developed at optimal and suboptimal temperature. Genotypes of different origin were compared to determine the conditions necessary to apply chlorophyll fluorescence analysis as a selection method to improve cold tolerance of photosynthesis. The validity of the method was demonstrated by the improvement in the photosynthetic capacity of hybrids between F<sub>3</sub> inbreeding families grown at suboptimal temperature.

## Materials and methods

### Plant material

Six *Zea mays* L. lines used in the experiments consisted of two cold-tolerant inbreds of European origin, Z7 and Z15, the hybrid between these two tolerant lines (Z7 × Z15), two cold-sensitive inbreds of tropical origin, Penjalinan (PENJ) and CM109, and the hybrid between the two sensitive lines (PENJ × CM109). The seedlings were grown in growth chambers (Conviron PGW36, Winnipeg, Canada) in 1.0 l pots in a soil/sand mixture (10:1, v/v) under a 12 h photoperiod (450 μmol photons m<sup>-2</sup> s<sup>-1</sup>), at a relative humidity of 60/70% (day/night). The plants were first grown at 25/22 °C (day/night) for 5 d and then grown at 25/22 °C or at 15/13 °C until full development of the third leaf. The plants were watered and fertilized with half-strength Hoagland nutrient solution as required. All measurements were performed on the fully expanded third leaves.

### Measurements of photosynthesis

O<sub>2</sub> evolution was measured on leaf segments with a leaf disc electrode unit (LD2/2, Hansatech, King's Lynn, UK) in 5% CO<sub>2</sub> at 15 °C or 25 °C. Light was provided by a Björkman lamp (LS2, Hansatech, King's Lynn, UK). The rate of photosynthetic O<sub>2</sub> evolution was obtained by subtracting the rate of dark respiration from the oxygen evolution rate in the light.

The net rate of CO<sub>2</sub> assimilation was measured on the middle part of fully developed third leaves using a portable Li-Cor 6200 apparatus (Li-Cor, Lincoln, USA) under growing conditions.

### Determination of chlorophyll content

The content of chlorophyll *a* + *b* was determined from 1 cm diameter leaf discs extracted with 2 ml 80% acetone (Arnon, 1949).

### Chlorophyll fluorescence measurements

Chlorophyll *a* fluorescence was recorded with a pulse amplitude modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany). The photochemical quenching (*q<sub>p</sub>*), the efficiency of excitation energy capture by open PSII reaction centres (*F<sub>v</sub>'*/*F<sub>m</sub>'*) and the quantum yield of electron transport at photosystem II (PSII),  $\Phi_{\text{PSII}}$ , were determined (Genty *et al.*, 1989). *F<sub>o</sub>'*, which was used for the determination of *q<sub>p</sub>* and *F<sub>v</sub>'*/*F<sub>m</sub>'* (Bilger and Schreiber, 1986), was measured after switching off the actinic light and simultaneously applying 3 s of far red light (735 nm, 15 W m<sup>-2</sup>).

### Response of chlorophyll fluorescence parameters to decreasing temperature

Intact plants were dark-adapted in a growth chamber at 25 °C for 15 min. The middle part of the third leaf was fixed on a leaf clip (2030-b, Walz, Effeltrich, Germany) which allows to record the temperature of the leaf. The maximum quantum efficiency of PSII primary photochemistry (*F<sub>v</sub>*/*F<sub>m</sub>*) was then determined by application of a 1 s saturation flash (>10 000 μmol photons m<sup>-2</sup> s<sup>-1</sup>). The leaves were then exposed to actinic illumination (125 μmol photons m<sup>-2</sup> s<sup>-1</sup>) of red light (655 nm) provided by the light-emitting diode array of the PAM fluorometer. After 15 min adaptation to light, the plant was cooled gradually from 25 °C to 2.5 °C at a rate of 0.17 °C min<sup>-1</sup> while saturation flashes were applied every 75 s. The recorded data for each leaf were pooled by 1 °C intervals.

### Response of $\Phi_{\text{PSII}}$ to increasing light intensity

Intact plants adapted to the dark (15 min at room temperature) were placed in a growth chamber at 25, 15 or 6 °C. The middle part of the third leaf was fixed on the PAM leaf clip and the plant was left in the dark until the leaf temperature and ambient temperature were the same. After *F<sub>v</sub>*/*F<sub>m</sub>* determination, the leaf was exposed to the lowest actinic illumination (50 μmol photons m<sup>-2</sup> s<sup>-1</sup>) for 20 min before  $\Phi_{\text{PSII}}$  was determined by five successive saturation flashes at 1 min intervals. Thereafter, the leaf was adapted for 10 min to each light intensity (up to 1000 μmol m<sup>-2</sup> s<sup>-1</sup>) before determination of  $\Phi_{\text{PSII}}$ . Five successive measurements were made to test if  $\Phi_{\text{PSII}}$  had reached a steady-state value since the last change of actinic light intensity. The fact that  $\Phi_{\text{PSII}}$  did not change significantly between the first and the fifth measurement (data not shown) indicated that 10 min adaptation was sufficient to reach a stable  $\Phi_{\text{PSII}}$  value under all light and temperature conditions tested. Actinic light was provided by a KL1500 lamp (Schott, Mainz, Germany) through an optic fibre. For each light intensity,  $\Phi_{\text{PSII}}$  was estimated from the average of the five measurements.

### Maize families selected from breeding populations for high $\Phi_{\text{PSII}}$ at low temperature

A Swiss dent and a Swiss flint corn type breeding population were used for the selection procedure. First, 40 seeds of each population were sown in the field and plants of each population were intercrossed yielding 31 ears of flint and 38 ears of dent. Twelve plants of each of these single ear progenies were grown in a growth chamber at suboptimal temperature as described for the model genotypes.  $\Phi_{\text{PSII}}$  was measured on the third fully expanded leaf at 6 °C and an actinic illumination 60 μmol

photons  $\text{m}^{-2} \text{s}^{-1}$ . The average value of the measurements of the 12 plants enabled us to determine the eight families in each population with the highest  $\Phi_{\text{PSII}}$ . These families were used for further selection. Forty plants of each of these selected  $F_1$  families were grown and analysed under the same conditions in order to select the best four individuals in each family. These plants were transplanted to the field and self-pollinated, yielding one to four  $F_2$  ears, depending on the family. Fifteen plants of each progeny were then tested as described above to determine the best four  $F_2$  families in each population and the best progeny within these families. Forty plants of each of the selected  $F_2$  families were then tested to determine the best five individuals. These were transferred to the field and self-pollinated. Ten plants of each  $F_3$  ear were then tested to determine the best progeny of each family. These were denominated H1, H2, H3, and H4, according to their average  $\Phi_{\text{PSII}}$  value (in decreasing order). Forty plants from each of these selected  $F_3$  families were then analysed as described above to determine the best five individuals, which were then transferred to the field. Hybrids between the different families were produced to compare with the original breeding populations. Hybrids were preferred to inbreds to avoid the undesirable effects of inbreeding depression.

## Results

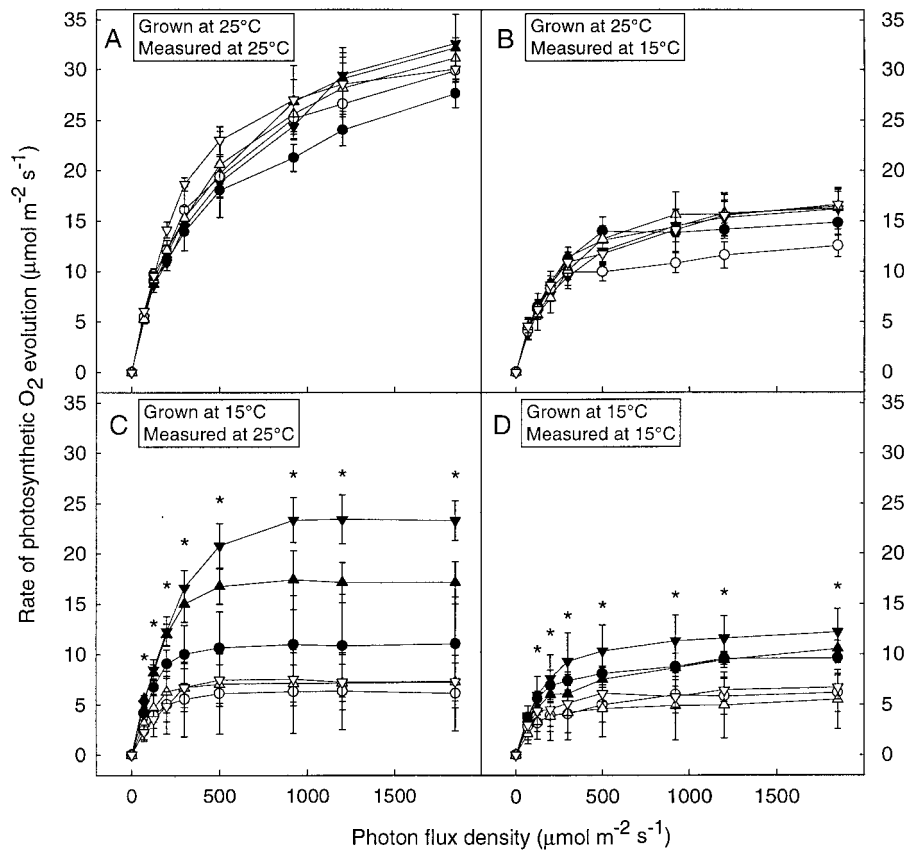
The photosynthesis of all lines was strongly affected by temperature (Fig. 1). The rate of photosynthetic oxygen evolution in leaves developed at 25 °C was greatly reduced by decreasing the measuring temperature from 25 °C to 15 °C. There was no clear genotypic difference in leaves grown at 25 °C, with the exception that the cold-sensitive line, Penjalinan, had a slightly lower photosynthetic capacity than the other lines when the measurements were performed at 15 °C (Fig. 1B). Photosynthetic capacity was lowest in leaves developed and measured at 15 °C (Fig. 1D). Under these conditions, the photosynthetic capacity of the three tolerant genotypes was significantly higher than the photosynthetic capacity of the three sensitive genotypes. Furthermore, when the measurement temperature was increased to 25 °C the photosynthetic capacity of two of the tolerant genotypes increased, in contrast to the sensitive genotypes (Fig. 1C). Tolerant genotypes were also characterized by a higher chlorophyll content than sensitive genotypes at both growth temperatures (Table 1), but the reduction in the chlorophyll content in response to low temperature was more pronounced in the sensitive genotypes (–54% on average) than in the tolerant genotypes (–24% on average). While the genotypic difference in the chlorophyll content correlated with the difference in the photosynthetic capacity in 15 °C leaves, this was not the case in 25 °C leaves (Fig. 1). Growth at low temperature also led to a slight, but significant decrease in the maximum quantum efficiency of PSII primary photochemistry ( $F_v/F_m$ , Table 1), indicative of chronic photoinhibition. However, there was no significant difference in  $F_v/F_m$  between tolerant and sensitive genotypes at both growth temperatures.

Figure 2 shows the effect of gradual cooling at moderate light intensity on the chlorophyll fluorescence characteristics of the leaves. The quantum yield of electron transport ( $\Phi_{\text{PSII}}$ ) was clearly strongly inhibited at temperatures below 10 °C in leaves developed at 25 °C and at 15 °C. In leaves grown at 25 °C, this reduction was associated with a decrease in photochemical quenching ( $q_P$ ) as well as in the efficiency of open reaction centres ( $F_v/F_m$ ). Sensitive and tolerant genotypes differed only slightly in their response to temperature when the leaves were developed at 25 °C. In contrast, tolerant and sensitive genotypes could be clearly separated on the basis of  $F_v/F_m$  and  $\Phi_{\text{PSII}}$  in leaves grown at 15 °C at temperatures below 8–10 °C. When compared with leaves grown at 25 °C, leaves grown at 15 °C were characterized by a lower efficiency of open reaction centres ( $F_v/F_m$ ) at all temperatures and by higher  $q_P$  values at temperature below 10 °C. The response of  $\Phi_{\text{PSII}}$  was further investigated by exposing leaves to increasing illumination at 25, 15 and 6 °C (Fig. 3). As in the previous experiment, measurements of leaves grown at 25 °C revealed little difference between genotypes under all measuring conditions. Increasing the light intensity resulted in a decrease in  $\Phi_{\text{PSII}}$  which was accelerated at low temperature. For a given temperature,  $\Phi_{\text{PSII}}$  was always lower in leaves grown at 15 °C than in leaves grown at 25 °C. Tolerant and sensitive genotypes, developed at 15 °C, could be separated best on the basis of  $\Phi_{\text{PSII}}$  at light intensities below 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and at a measuring temperature of 6 °C.

This promising result led us to determine whether  $\Phi_{\text{PSII}}$  measured at low temperature could be used to select for the maintenance of photosynthetic capacity of plants developed at suboptimal temperature by breeding using the procedure described in Materials and methods. The result in Table 2 shows that this is the case for five out of the eight hybrids between selected  $F_3$  families, the best being the dent H2  $\times$  H1 cross which showed a 31% increase of photosynthesis when compared with the original dent population when the plants were grown at suboptimal temperature. Two of the flint crosses also showed a significant increase in photosynthesis when compared with the original flint population when the plants were grown under optimal temperature. Three of the five crosses with improved photosynthetic capacity at suboptimal growth temperature also showed significantly higher chlorophyll contents than the original populations (Table 2).

## Discussion

The three tolerant lines had higher rates of photosynthesis than the three sensitive lines when grown at suboptimal temperature, showing that there is much genetic variability within the *Zea mays* species as far as the adaptation of the photosynthetic apparatus to low growth temperature is concerned. In addition, the observation that the



**Fig. 1.** The effect of temperature and light intensity on the photosynthetic oxygen evolution of the third leaves of cold-tolerant (closed symbols) and cold-sensitive (open symbols) maize genotypes developed at 25 °C (A, B) and 15 °C (C, D) and measured at 25 °C (A, C) or 15 °C (B, D). (●) Z7; (▲) Z15; (▼) Z7 × Z15; (○) PENJ; (△) CM109; (▽) PENJ × CM109. Values are means (±SD) of five plants. Stars indicate light intensities where pooled tolerant genotypes differed from pooled sensitive genotypes in a *t*-test comparison with *P* < 0.001.

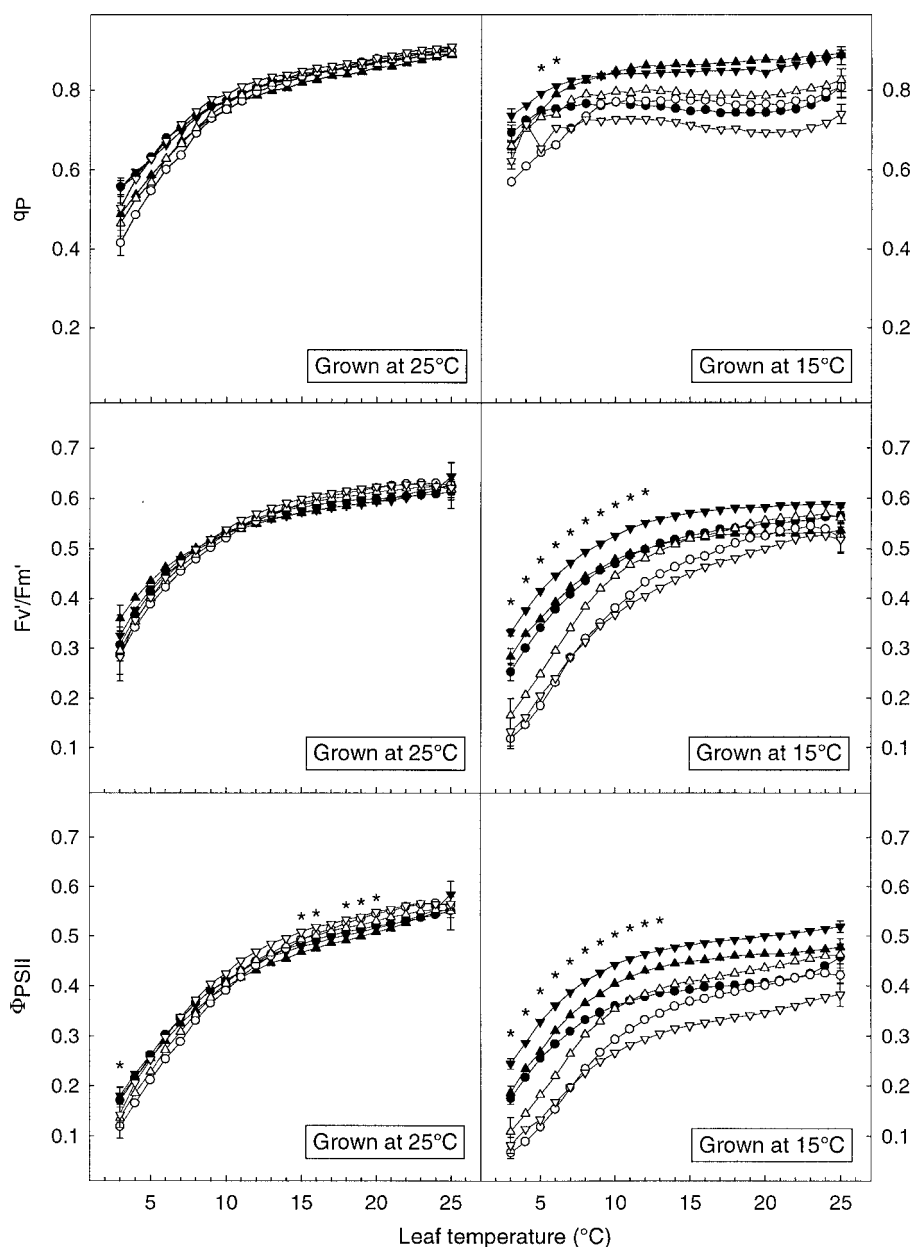
**Table 1.** The maximum quantum efficiency of PSII primary photochemistry ( $F_v/F_m$ ) and the chlorophyll a + b content in maize leaves developed at 25 °C or 15 °C

CT: pooled tolerant genotypes; CS: pooled sensitive genotypes. Values are means (±SD) of five plants. Stars indicate *t*-test comparison between CT and CS for a given growth temperature. \*\*\*: *P* < 0.001, NS not significant at *P* < 0.05.

Genotype	Parameter		Chl a + b (μmol m <sup>-2</sup> )	
	$F_v/F_m$			
	Grown at 25 °C	Grown at 15 °C	Grown at 25 °C	Grown at 15 °C
Z7	0.780 ± 0.010	0.668 ± 0.014	491 ± 43	319 ± 48
Z15	0.785 ± 0.007	0.709 ± 0.016	598 ± 49	512 ± 65
Z7 × Z15	0.784 ± 0.011	0.713 ± 0.017	516 ± 22	388 ± 20
PENJ	0.780 ± 0.006	0.705 ± 0.020	351 ± 49	171 ± 39
CM109	0.794 ± 0.004	0.708 ± 0.015	464 ± 39	189 ± 51
PENJ × CM109	0.780 ± 0.008	0.722 ± 0.020	483 ± 60	195 ± 25
CT	0.783 ± 0.010	0.696 ± 0.026	556 ± 83	399 ± 91
CS	0.785 ± 0.009 NS	0.712 ± 0.020 NS	422 ± 83***	185 ± 42***

photosynthesis of leaves of two of the tolerant genotypes, grown at 15 °C, increased substantially when the temperature was switched from 15 °C to 25 °C (Fig. 1C, D) is of particular importance for plants growing under natural conditions. It suggests that tolerant genotypes may benefit immediately from a warmer temperature after a prolonged

period of low temperatures. It is likely that they could better meet the increased demand for carbohydrates than the sensitive genotypes in order for growth to resume with increasing temperature. This hypothesis is in agreement with the observation that the two inbreds, Z7 and Z15, showed higher relative growth rates than the two



**Fig. 2.** The effect of decreasing temperature on chlorophyll fluorescence parameters of maize leaves. Values are means ( $\pm$ SD) of five plants. For clarity, error bars are shown only at the ends of the curves. See Fig. 1 for legend.

sensitive inbreds, Penjalinan and CM109, when grown under cool conditions in the field (Verheul *et al.*, 1996).

The poor photosynthetic performance of the sensitive lines grown at 15°C was associated with a chlorophyll deficiency (Table 1) (Haldimann, 1998). The chloroplast development of the sensitive genotypes may be particularly sensitive to low temperature or, alternatively, the pigments of sensitive genotypes might be destroyed because of a high sensitivity to oxidative stress induced by low temperature.

The cold tolerance of the genotypes was not correlated to the maximum efficiency of PSII photochemistry ( $F_v/F_m$ ,

Table 1) either in leaves grown at 25°C nor in leaves grown at 15°C, even though growth at suboptimal temperature led to a slight decrease of  $F_v/F_m$ . However, using  $F_v/F_m$  to select for tolerance to severe chilling may be a good approach since there is a great genetic variation in tolerance to low temperature-induced photoinhibition in maize (Dolstra *et al.*, 1994). The fact that the tolerant line Z7 had the lowest  $F_v/F_m$  value of all the lines when grown at 15°C but a relatively high photosynthetic capacity, indicates that moderate photoinhibition hardly affects the photosynthetic rate at high light.

Decreasing leaf temperature induced a decrease in

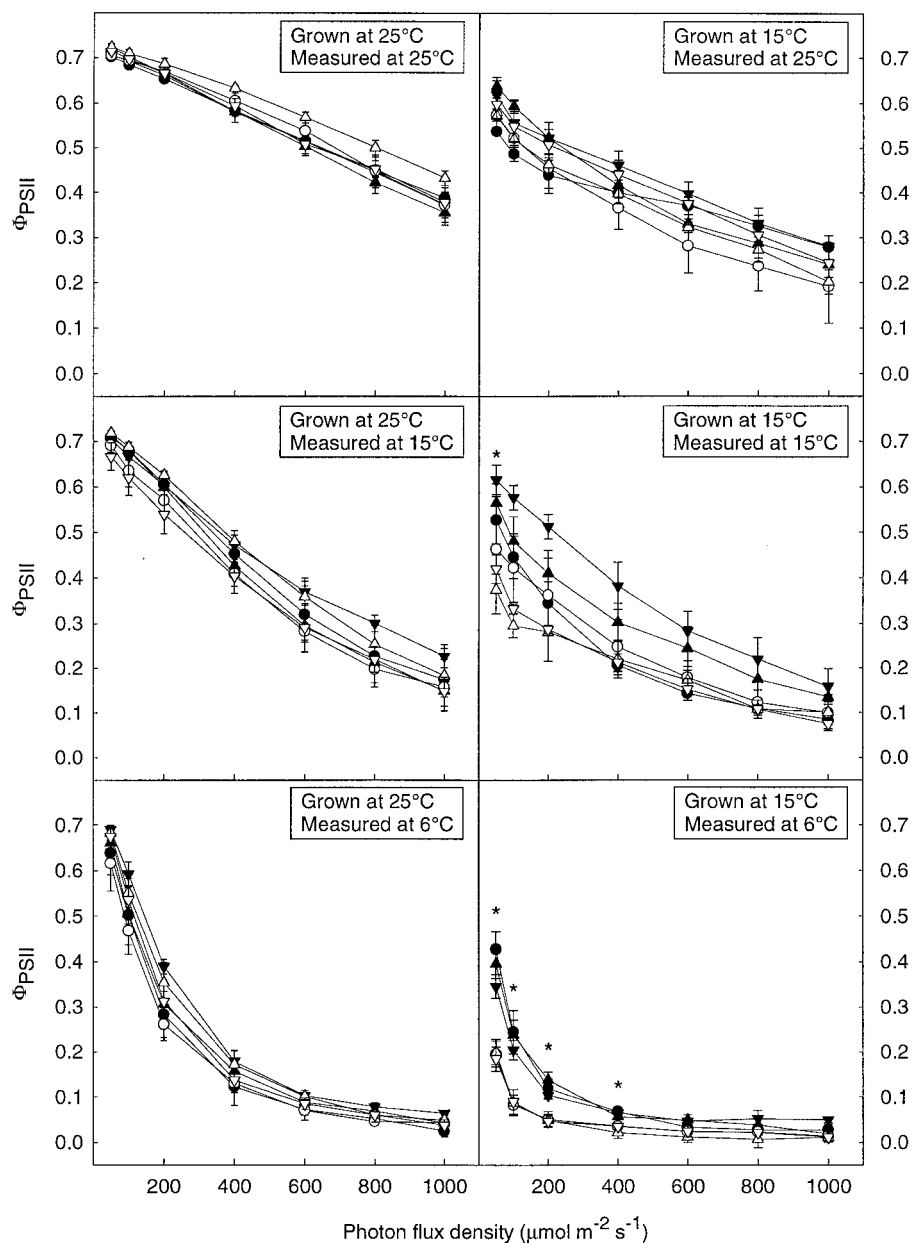


Fig. 3. The effect of increasing light intensity on  $\Phi_{\text{PSII}}$  of maize leaves at 6, 15 and 25 °C. Values are means ( $\pm$ SD) of five plants. See Fig. 1 for legend.

photosynthetic electron transport activity, resulting in a clear difference between tolerant and sensitive genotypes in leaves developed at suboptimal temperature (Fig. 2). Since all genotypes behaved similarly when the leaves were developed at optimal temperature, the genotypic difference must be related to adaptation mechanisms induced by suboptimal growth temperature. Constitutive differences probably also exist in maize. Genotypic variation in the response of  $q_p$  to low temperature in leaves developed at 25 °C has been reported (Havaux, 1987). Interestingly, leaves of all genotypes grown at 15 °C maintained higher  $q_p$  value than leaves grown at 25 °C in response to gradual cooling (Fig. 2). This suggests that

protection mechanisms are present both in sensitive and in tolerant genotypes, allowing the leaves to prevent excessive reduction of PSII acceptors. It has been suggested that this feature is related to the high content of the xanthophyll zeaxanthin in maize leaves developed at suboptimal temperature (Haldimann *et al.*, 1995). Many researchers (see Demmig-Adams and Adams, 1996, for a review) reported that this pigment is related to excess energy dissipation as heat in the antennae. Thus, its presence in leaves developed at suboptimal temperature is probably also responsible for the reduced efficiency of excitation energy capture by open reaction PSII reaction centres ( $F_v/F_m$ ) when compared with leaves grown at

**Table 2.** The net rate of CO<sub>2</sub> assimilation (*A*) and chlorophyll a+b content (Chl a+b) of leaves of hybrids between F<sub>3</sub> maize lines bred for cold tolerance (H1 to H4) as well as of the original populations from which they are derived (O)

Measurements were performed under the growth conditions (25 °C or 15 °C, light intensity: 450 µmol photons m<sup>-2</sup> s<sup>-1</sup>). Values are means (±SD) of 6 or 18 plants for the hybrids and the original populations, respectively. Stars indicate that, for a given growth temperature, the selected hybrid was significantly different from the original population in a *t*-test comparison. \*: *P*<0.05; \*\*: *P*<0.01; \*\*\*: *P*<0.001; NS: not significant at *P*<0.05.

Cross or population	Parameter			
	<i>A</i> (µmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )		Chl a+b (µmol m <sup>-2</sup> )	
	Grown at 25 °C	Grown at 15 °C	Grown at 25 °C	Grown at 15 °C
Flint				
H1 × H2	19.64 ± 0.86*	16.58 ± 0.98***	508 ± 12***	450 ± 46*
H2 × H1	18.85 ± 1.64 NS	16.27 ± 1.43**	412 ± 49 NS	499 ± 61**
H3 × H4	20.03 ± 1.52*	15.52 ± 1.89*	443 ± 42 NS	409 ± 46 NS
H4 × H3	19.23 ± 1.97 NS	16.47 ± 1.49***	389 ± 15 NS	430 ± 51 NS
O	18.15 ± 1.66	12.96 ± 2.02	385 ± 67	362 ± 87
Dent				
H1 × H2	19.41 ± 1.13 NS	14.40 ± 1.67 NS	421 ± 30 NS	314 ± 52 NS
H2 × H1	19.67 ± 1.55 NS	16.90 ± 1.21***	397 ± 25 NS	426 ± 61***
H3 × H4	19.12 ± 0.88 NS	12.94 ± 0.72 NS	448 ± 62*	332 ± 48 NS
H4 × H3	18.73 ± 0.93 NS	13.39 ± 0.50 NS	384 ± 45 NS	331 ± 48 NS
O	19.23 ± 1.74	12.86 ± 1.58	391 ± 44	298 ± 44

optimal temperature (Fryer *et al.*, 1995). Furthermore, the difference in the behaviour of  $\Phi_{\text{PSII}}$  in the genotypes (Fig. 2) is largely due to a difference of  $F_v'/F_m'$ , suggesting that xanthophyll cycle pigments might be related to the genotypic difference. The recent observation that, when leaves are grown at suboptimal temperature, sensitive genotypes accumulate more zeaxanthin than tolerant genotypes (Haldimann, 1998) supports this hypothesis. The greater capacity of the tolerant lines to maintain higher electron transport rates than sensitive lines at low temperature (indicated by  $\Phi_{\text{PSII}}$ ) does not necessarily mean that the quantum yield of carbon fixation of the tolerant lines was superior under these conditions, since the linear relation between  $\Phi_{\text{PSII}}$  and the quantum yield of CO<sub>2</sub> fixation usually observed in maize (Genty *et al.*, 1989; Massacci *et al.*, 1995) can be deviated in leaves developed at low temperature in the field (Fryer *et al.*, 1998).

In the experiment presented in Fig. 2, the lowest temperature was reached after around 2 h of gradual cooling, long enough for important changes within the photosynthetic apparatus to occur, such as the conversion of xanthophyll cycle pigments (Leipner *et al.*, 1997). These changes may have significant effects on chlorophyll fluorescence quenching. For this reason, the response of  $\Phi_{\text{PSII}}$  to temperature was further investigated by exposing leaves to light at 6, 15 or 25 °C (Fig. 3). The results of the measurements made at 6 °C clearly confirm the genotypic difference observed in Fig. 2. Furthermore, the response of  $\Phi_{\text{PSII}}$  to increasing light intensity at 15 °C and 25 °C provides further information about the nature of this difference. The fact that leaves of tolerant and sensitive genotypes grown at 15 °C, behaved similarly at these two temperatures even under high illumination indicates that the cause of the genotypic difference visible at 6 °C is not

expressed in response to excess energy *per se*, but is specific to low leaf temperature.

Although the cause of the genotypic difference remains to be found, the clear difference between tolerant and sensitive genotypes, detected when leaves grown at 15 °C were exposed to low illumination (<200 µmol photons m<sup>-2</sup> s<sup>-1</sup>) at 6 °C (Fig. 3), suggests that  $\Phi_{\text{PSII}}$  is a useful criterion for differentiating between maize plants with different cold tolerance of photosynthetic capacity.  $\Phi_{\text{PSII}}$  of *Triticum* species of different origin has also been reported to differ substantially at low temperature (Rekika *et al.*, 1997) and could be used to discriminate between wheat cultivars that differ in drought resistance (Flagella *et al.*, 1996). Because  $\Phi_{\text{PSII}}$  is directly related to the rate of electron transport, it might be a useful tool for selecting for resistance to different types of stress which inhibit photosynthesis. However, to the best of our knowledge, the use of  $\Phi_{\text{PSII}}$  as a selection tool to improve the stress tolerance of photosynthesis through breeding has not yet been reported. Thus, it was interesting to determine whether its use in a breeding programme would improve the cold tolerance of photosynthesis in maize. The result, summarized in Table 2, shows that the use of  $\Phi_{\text{PSII}}$  in breeding programmes can substantially increase the photosynthetic capacity of maize plants developed at suboptimal temperature. The hybrids of F<sub>3</sub> families are already different to the original population, suggesting that only a limited number of genes are involved in the trait (F<sub>3</sub> are only F<sub>2</sub> inbreds because F<sub>1</sub> were hybrids). These traits might be dominant, or the selection method may have led to the independent selection for the same traits in the different families.

The relatively small number of plants used at each selection step (maximum 40 for a selected ear, see

Materials and methods) suggest that the method is very efficient, assuming that the genetic variation within the original population is large enough. Indeed, the variation in the photosynthesis of leaves developed at suboptimal temperature seems larger in the original flint population than in the dent population (see SD values in Table 2). This may explain why the selection programme was more efficient with the flint population than with the dent population.

In several cases, selection also led to a significant increase in the leaf chlorophyll content of plants grown at suboptimal temperature compared with the original populations (Table 2). This is not very surprising in view of the pattern obtained with the model genotypes (Table 1). An increase in the tolerance of photosynthetic capacity may often occur with a concomitant increase in the ability to maintain high chlorophyll content at suboptimal growth temperature.

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